

# RATE OF BREAKING AND SIZE OF THE "HALVES" OF THE ARBACIA PUNCTULATA EGG WHEN CENTRIFUGED IN HYPO- AND HYPERTONIC SEA WATER

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## PROBLEM

*Arbacia punctulata* eggs, when centrifuged in a mixture of sea water and isosmotic cane sugar solution used in the proper proportion to keep the eggs suspended during centrifugation, break quite uniformly into "halves" of definite size (E. N. Harvey, 1931; E. B. Harvey, 1932-1941). The question arises as to whether the eggs break more or less readily in hypotonic solutions than in sea water, and whether the relative size of the two "halves" remains the same, that is, whether the extra water is distributed equally in the two halves. It has been shown by Lucké (1932b, 1940) that when the eggs are broken into halves in sea water first and the halves are then placed in hypotonic sea water, both halves swell but the heavy (red) half swells a little less than the light (white) half owing to the presence of more of the osmotically inactive material (yolk granules) in this (red) half. The present problem is concerned with centrifuging the eggs *after* they have been swollen in hypotonic sea water. It has been shown (E. B. Harvey, 1941) that the rate of breaking and the relative size of the two halves varies with the amount of centrifugal force used. With a force of  $10,000 \times g$ , which I have taken as a standard force throughout my experiments, the white (centripetal) half is slightly larger than the red (centrifugal) half. With a greater force, the red half is larger while the white half is correspondingly smaller. With a smaller force, the red half is smaller than with greater forces, and the white half correspondingly larger. In the present experiments, therefore, a uniform standard force was used,  $10,000 \times g$ .

The size of the halves obtained by centrifuging the eggs in hypo- and hypertonic sea water and subsequently returning them to normal sea water was also studied, in order to determine how the normal water balance was regained.

## METHODS

Before centrifuging, the eggs from one female were kept for a half hour in 60 per cent, 80 per cent, 100 per cent (control) and 125 per cent sea water, a sufficient time for them to attain equilibrium with the medium. Eggs kept for six hours in the solutions showed no appreciable further change in size. The sugar solutions added to the sea water to keep the eggs suspended during centrifugation, were made up of the same tonicity as the hypo- and hypertonic sea water. The four tubes containing 60 per cent, 80 per cent, 100 per cent (control) and 125 per cent sugar-sea water solutions were all centrifuged at the same time in each experiment;

each tube contained one part of the egg suspension to three parts of the corresponding sugar solution, this being the proper proportion to keep the eggs suspended and free to break during centrifugation. The unbroken eggs in all the tubes come to lie at the same level, so that they are all subjected to the same centrifugal force which, of course, varies with the radial distance of the layer of eggs from the axis of the centrifuge, according to the equation  $F = .04 \times R (= \text{radius in cm.}) \times (\text{R.P.S.})^2$ . It is necessary to use the eggs from only one animal for one experiment since there is considerable variability in size, segregation of granules and ease of breaking in eggs from different females, but those from one female are remarkably constant in this respect. The eggs were centrifuged for three to six minutes at  $10,000 \times g$ , according to the ease of breaking of the particular batch of eggs, and were then placed in dishes of sea water of the corresponding tonicity. The measurements were made with an ocular micrometer and checked in several experiments with a filar micrometer; the figures are accurate to about  $0.6 \mu$ . The measurements recorded are the average of ten cells, made with an optical equipment giving a magnification of 400 times; the eggs lay free in the media in Syracuse watch glasses.

The experiments were performed many times with the same general results. The data obtained in a typical experiment are given in Table I A, B, C. The same eggs were used throughout the experiment.

## RESULTS

### *Rate of breaking* (Table I A)

The rate of breaking into halves may be judged by the number of broken eggs in comparison with the number of whole, unbroken eggs obtained after centrifuging for a definite time with a definite force. When tubes containing suspensions of eggs in a 60 per cent sea water-sugar medium, 80 per cent, 100 per cent (control) and 125 per cent were centrifuged at the same time, usually for four minutes at  $10,000 \times g$ , the degree of breaking increased in the order named. In most of the experiments, practically all the eggs were broken in the 125 per cent medium while very few were broken in the 60 per cent medium. An average experiment (Table I A), gave 10 per cent of the eggs broken in the 60 per cent medium, 20 per cent in the 80 per cent medium, 70 per cent in normal sea water and practically all in the 125 per cent medium. In an experiment where only 50 per cent of the eggs were broken in the 125 per cent medium, none were broken in the 60 per cent medium. The eggs break, therefore, less readily in hypotonic sea water, and more readily in hypertonic sea water, than they do in normal sea water.

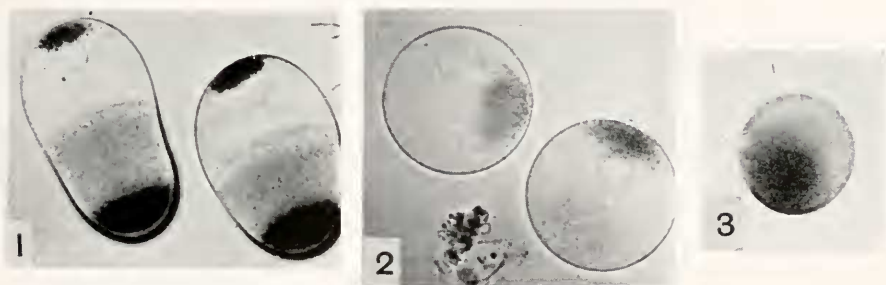
### *Size of the halves* (Table I A; Photographs, Plate I)

When eggs are swollen in hypotonic sea water or shrunken in hypertonic sea water and then centrifuged, the increase and decrease in size is almost entirely in the white halves, the red halves being nearly the same size as those centrifuged in

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## PLATE I

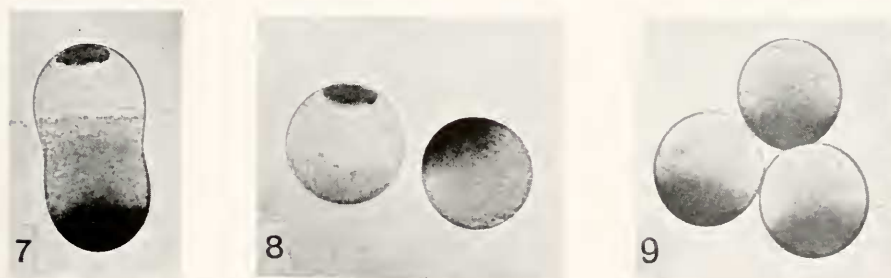
Photographs of living *Arbacia punctulata* eggs centrifuged in hypo- and hypertonic sea water, and the controls in normal sea water, and the halves into which they break with a force of  $10,000 \times g$  for four minutes. Magnification approximately  $275 \times$ , all magnified exactly the same.



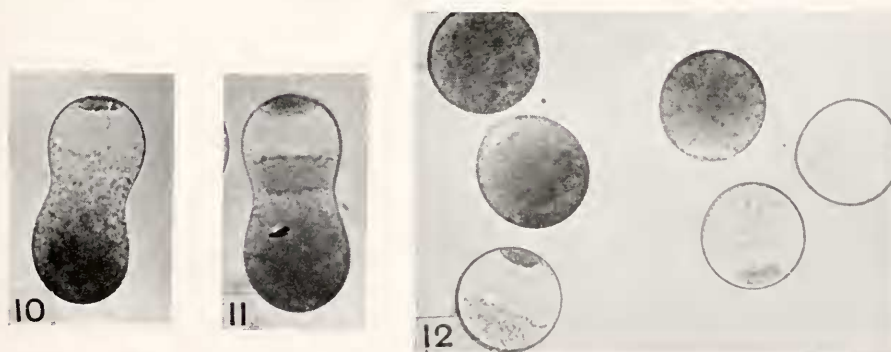
60%



80%



Control 100%



125%

PLATE I

normal sea water (Table I A). When the eggs are centrifuged in 60 per cent sea water, the white halves are very much larger than those obtained in the control (100 per cent sea water), whereas the red halves are only slightly larger than in the control (Cf. Photographs 2, 3 with 8, 9). When centrifuged in 80 per cent sea water, the white halves are somewhat larger than in the control, the red halves almost the same size (Photographs 5, 6). When centrifuged in hypertonic sea

TABLE I

Sea water	Whole egg		White half		Red half		Nucleus		Per cent broken
	Diam. $\mu$	Vol. $\mu^3$	Diam. $\mu$	Vol. $\mu^3$	Diam. $\mu$	Vol. $\mu^3$	Diam. $\mu$	Vol. $\mu^3$	
<i>A. Eggs in hypo- and hypertonic sea water, then centrifuged</i>									
60%	82.4	(292,900)	70.4	(182,700)	58.0	(102,200)	16.0	(2,145)	10%
80%	74.9	(220,000)	62.1	(125,400)	56.3	(93,400)	12.8	(1,098)	20%
100%	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)	11.5	(796)	70%
125%	66.6	(154,700)	51.7	(72,360)	53.8	(81,540)	9.6	(382)	98%
<i>B. Recovery in 100 per cent sea water</i>									
60%-100%	72.0 <sup>1</sup>	(195,400)	59.2	(108,600)	55.7	(90,480)	11.2	(736)	
80%-100%	72.0 <sup>1</sup>	(195,400)	59.2	(108,600)	54.7	(86,170)	11.5	(796)	
100%	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)	11.5	(796)	
125%-100%	72.0 <sup>1</sup>	(195,400)	56.3	(93,940)	57.6	(100,060)	11.2	(736)	
<i>C. Eggs centrifuged in sea water, then placed in hypo-hypertonic sea water</i>									
100%-60%	82.4	(292,900)	67.4	(160,300)	63.4	(133,400)			
100%-80%	74.9	(220,000)	61.8	(123,600)	57.2	(97,990)			
100%	72.0	(195,400)	59.0	(107,500)	56.0	(91,950)			
100%-125%	66.6	(154,700)	54.4	(84,300)	51.2	(70,300)			
<i>D. Lucké's (1932b) <sup>2</sup> mean values for C (above)</i>									
100%-60%	(84.6)	317,380	(69.5)	175,560	(63.9)	136,700			
100%-80%	(80.6)	274,020	(66.3)	152,320	(61.7)	123,060			
100%	(72.2)	197,440	(59.2)	108,600	(55.7)	90,680			

<sup>1</sup> These are not actual measurements because of lack of time to measure these in the same experiment as the rest. The return to normal size is approximately perfect, as determined in other experiments and as found also by Lucké and co-workers, who publish their measurements (1931a, p. 402).

<sup>2</sup> Only the volumes are given by Lucké; the diameters are calculated from the volumes.

water (125 per cent) the white halves are much smaller than in the control, the red halves about the same size as the controls, sometimes larger, sometimes a little smaller; the white halves are now in most experiments smaller than the red halves (Photograph 12); in a few experiments they were the same size. In the controls, the white halves were always considerably larger than the red halves (Photographs 8, 9).

Unusual batches of eggs occur occasionally, as noted in previous papers

(1936, 1941), in which, when centrifuged in normal sea water with the standard force, the red half is very small, and the white half correspondingly very large, in the ratio of 8 : 1 by volume. When such batches of eggs are centrifuged in 80 per cent sea water, the halves are of approximately the same relative size as in normal batches, in the ratio of 4 : 3 by volume, as noted previously (1941). When the unusual batches are centrifuged in hypertonic sea water, on the other hand, the relative inequality in the two halves remains; that is, the red halves are very small.

*Stratification of whole eggs, and content of halves* (Photographs, Plate I)

As would be expected, the granules pack much more when the eggs are centrifuged in hypotonic media than in normal sea water (Photographs 1, 4, 7). The packing of the granules takes place to such an extent in the 60 per cent sea water that the clear layer is very large and usually the white halves (Photograph 2) are almost entirely free of granules, all of them having been thrown down into the red half, although, as stated above, this red half is not much larger than the red half obtained in normal sea water which contains none of the mitochondria and only part of the yolk. This can be beautifully demonstrated in eggs stained with the vital dye, methyl green, which selectively stains the mitochondria. The purple-staining mitochondria are all in the red half. In the whole egg centrifuged in 60 per cent sea water, the mitochondrial layer is very thin, being spread over a greater area. The pigment granules are so well packed in the hypotonic solutions that the line of demarkation between yolk and pigment is very sharp, much more so than in eggs centrifuged in normal sea water. When the eggs are centrifuged in 60 per cent sea water, many of the white halves and also the upper portion of the whole eggs containing the clear layer burst soon after centrifugation; the red halves and red portion of the whole egg remain intact. This bursting is probably due to the thinness of membrane which presumably decreases in thickness as the area it covers increases.

When centrifuged in hypertonic sea water, the clear layer is small, the mitochondrial layer very thick, being spread over a small area, and in many cases is very well marked (Photograph 11). The white half is thus quite granular. The pigment is not well separated from the yolk, there being no clear line of demarkation. It is obvious from Photograph 11, that it is in general not accurate to speak of "well-stratified" eggs, since they may be well-stratified with respect to the mitochondria and poorly stratified with respect to the pigment and yolk. Many batches of eggs occur, in which, when centrifuged in normal sea water, the mitochondrial layer is indistinguishable while the yolk and pigment layers are well formed. In typical batches of eggs, however, the stratification in normal sea water is intermediate between that obtained in hypotonic sea water and in hypertonic sea water (Photograph 7).

*Recovery in normal sea water* (Table I B; Photographs, Plate II)

When whole normal eggs are swollen in hypotonic sea water or shrunken in hypertonic sea water, and are then returned to normal sea water, they return to normal size, as shown previously (for hypotonic) by Lucké and co-workers (1931a; 1932a). The same holds for centrifuged whole eggs. The two half-eggs



obtained by centrifuging an egg swollen in hypotonic sea water, shrink when returned to normal sea water, but not at all to the same extent. The white half loses a great deal of water, the red half very little (Photographs 13, 14). The two halves become of approximately the same size as the two halves obtained from a normal egg centrifuged in normal sea water (Photographs 8, 9). The loss of water from the white halves takes place exceedingly rapidly. Lucké and co-workers (1927; 1931b; 1932a) have called attention to the much more rapid shrinking than swelling in the case of whole eggs.

When whole normal eggs are shrunk in hypertonic sea water, and returned to normal sea water, they likewise regain their normal size. When eggs are centrifuged in a hypertonic solution (125 per cent), the two halves, as stated above, are of nearly equal size, the red half being in most batches a little larger than the white half (Photograph 12). When these halves are returned to normal sea water, they gain water in approximately the same amount and at the same rate, so that they both become slightly larger, but keep approximately the same size relative to each other (Photographs 15, 16, 17). The white halves never attain the size of the white halves centrifuged off in normal sea water. If the halves from the hypertonic sea water are placed in hypotonic sea water (60 per cent), they still swell approximately the same amount, the red halves being in the batch pictured a little larger than the whites (Photograph 18).

*Size of nuclei in hypo- and hypertonic sea water and their recovery in sea water*  
(Table I and Plate I)

Though not directly related to the problem under discussion, the size of the mature nucleus in hypo- and hypertonic sea water is of sufficient interest to be recorded here. The nucleus of a normal mature unfertilized *Arbacia* egg is difficult to measure because it is imbedded in granules. However, when the egg is centrifuged, the nucleus lies in the clear layer under the oil cap and can easily be observed and measured in both the whole egg and the white half. The increase in size in hypotonic sea water, and the decrease in hypertonic sea water is quite apparent in photographs (Plate I). The nucleus of the normal egg in sea water measures approximately  $11.5\ \mu$  in diameter; in 60 per cent sea water the diameter is  $16\ \mu$ , an increase to two and a half times its volume; in 80 per cent sea water the diameter is  $12.8\ \mu$ ; in 125 per cent sea water the diameter is  $9.6\ \mu$ , a decrease to about one half the volume of the normal nucleus (Table I A). The cell increases to about one and one half its volume in 60 per cent, and decreases to three quarters its volume in 125 per cent sea water. The percentage increase and decrease in volume of the nucleus is greater than the percentage increase and decrease in

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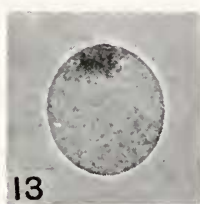
PLATE II

Photographs 13, 14. The two half-eggs obtained from centrifuging in hypotonic sea water (60 per cent, as shown in Photographs 2, 3) after their return to normal sea water.

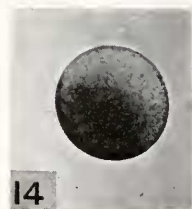
Photographs 15, 16, 17. The two half-eggs obtained from centrifuging in hypertonic sea water (125 per cent, as shown in Photograph 12) after their return to normal sea water; the same halves at 15-minute intervals. There was no further change in size in photographs taken several hours later.

Photograph 18. The two half-eggs obtained from centrifuging in hypertonic sea water (125 per cent, as shown in Photograph 12), after placing them in 60 per cent sea water.

Same magnification as in Plate I, approximately  $275\times$ .

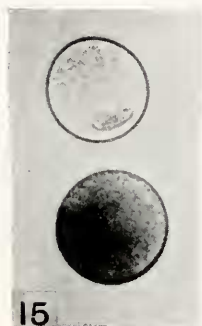


13



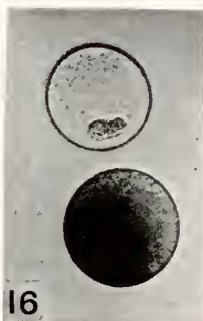
14

60% - 100%



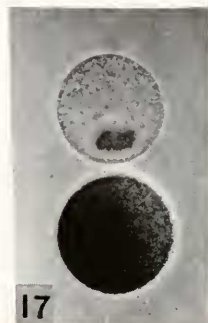
15

12.30 p.m.



16

12.45 p.m.



17

1.p.m.

125% - 100%



18

125% - 60%

volume of the egg. This may be due to changes in metabolism, or to a smaller amount of osmotically inactive material in the nucleus, or it may be due to a difference in the nuclear and cell membranes. Skowron and Skowron (1926)<sup>3</sup> have noted a similar volume difference of the germinal vesicle of the *Sphaerechinus granularis* egg in comparison with the egg itself when treated with hypotonic solutions. And Beck and Shapiro (1936) found the same thing true for the germinal vesicle of the starfish egg swollen in 80 per cent sea water.

The nuclei of the whole *Arbacia* eggs returned to normal sea water after hypo- and hypertonic sea water, regain their normal size (Table I B), but at a much slower rate than the egg itself.

In the case of the germinal vesicle of the immature *Arbacia* egg, Churney (1942) concluded that it swells and shrinks reversibly in anisotonic solutions, and acts as a better osmometer than the egg itself. Beck and Shapiro (1936) have likewise found that the germinal vesicle of the starfish egg shrinks and swells in the same sense as the cell, and they have called attention to the fact that the rate is slower for the nucleus than for the egg to attain equilibrium. The mature nucleus of the *Arbacia* egg, therefore, seems in all respects similar to the immature nucleus (germinal vesicle) of *Arbacia* and other sea urchins, and of the starfish, with regard to swelling and shrinking. This is of interest and not necessarily to be expected because (1) the membrane of the mature nucleus is a new formation after the polar bodies are given off and (2) the contents of the mature and immature nucleus are different both in morphological structure (e.g. the nucleolus) and in the amount of material present; the volume of the germinal vesicle of *Arbacia* is about 50 times that of the mature nucleus.

*Size of half-eggs obtained by centrifuging in normal sea water and then placing them in hypo- and hypertonic sea water (Table I C)*

The swelling of half-eggs obtained by centrifuging eggs in a 100 per cent sea water-sugar medium and then placing them in hypotonic sea water has been adequately studied by Lucké (1932b, 1940). He found that both the half-eggs swelled in hypotonic sea water, but that the white half swelled a little more than the red half because the latter contained more of the osmotically inactive material, which he estimates as 12 per cent. His mean values are given in Table I D. My figures agree fairly well with his. In Table I C, my figures are given for the swelling and shrinking of the same eggs and half eggs as used in the other parts of the same experiment (Table I A and B). One may thus compare, in the same batch of eggs, the allocation of excess water in the two halves obtained by centrifuging before and after treating with hypotonic sea water; and similarly for the extraction of water in hypertonic sea water.

## DISCUSSION

With a constant centrifugal force of  $10,000 \times g$ , *Arbacia* eggs break less rapidly in hypotonic sea water and more rapidly in hypertonic sea water than they do in normal sea water. The tension at the surface is increased with the increase

<sup>3</sup> These authors found no decrease in size of the germinal vesicle in hypertonic glucose ( $\Delta = 2.57$ ), though the cell shrank 57 per cent; this seems strange in view of their results for hypotonic glucose.



of surface area (Cole, 1932), so that if this factor alone were considered, the eggs should break less rapidly in hypotonic sea water, as they do. However, the densities of the half-eggs in comparison with the medium must also be considered, and these densities were not measured.

With regard to the size of the two halves, it is seen from the data presented that when *Arbacia* eggs are kept in hypotonic sea water and centrifuged in a similar medium, the egg breaks so that the light half is much larger than the heavy half, whereas in normal sea water it is only slightly larger. The excess water is distributed largely to the light half. Conversely, when the eggs are kept in hypertonic sea water and centrifuged in a similar medium the egg breaks so that the light half is usually slightly smaller than the heavy half. Much of the water is taken away from the light half. This is perhaps what is to be expected since it is the clear layer in the light half that contains most of the osmotically active material. Similarly, the large white halves from the hypotonic sea water lose much more water when returned to normal sea water than do the smaller, more granular red halves. On the other hand, when the eggs are centrifuged in hypertonic sea water, the granules are more evenly distributed between the two halves, now nearly equal in size. The clear layer is small, the white half is quite granular, and the granules in the heavy half are not well packed, so that there is probably more liquid (osmotically active) material present among these granules than is apparent to the eye. Thus, when these two halves are returned to normal sea water, they swell approximately the same amount.

It might be of interest to compare the results obtained with hypo- and hypertonic sea water with those previously obtained by changing the centrifugal force (1941; compare Plate I of the present paper with Plate I of the previous paper). A low force acts similarly to hypotonic sea water; the heavy granules are well segregated, the light half is much larger than the heavy half, and the egg breaks apart less readily. A high force acts similarly to hypertonic sea water; the heavy granules are not well segregated, the light half is smaller than the heavy half, and the egg breaks apart more readily. Perhaps one might conclude that when the heavy granules are well packed, whether by using a low force or by adding water, the granular half is smaller in comparison with the less granular, and the egg breaks more slowly.

#### SUMMARY

1. *Arbacia punctulata* eggs, when centrifuged with a force of  $10,000 \times g$ , break less readily in hypotonic sea water, and more readily in hypertonic sea water than in normal sea water.

2. When broken apart in hypotonic sea water, the white half is very much larger than the red half. The white half is much larger than the white half obtained by centrifuging in normal sea water, the red half only slightly larger than the red half obtained in normal sea water.

3. When broken apart in hypertonic sea water, the white and red halves are of almost equal size, the white half usually a little smaller than the red half. When broken apart in normal sea water, the white half is somewhat larger than the red half. The white half from the hypertonic sea water is much smaller than the white half from normal sea water, the red half nearly the same size.

4. When the halves obtained by centrifuging in hypotonic sea water are returned to normal sea water, they both lose water, but the white half to a much greater extent than the red half. They become of approximately the same size as though they had been centrifuged in normal sea water.

5. When the halves obtained by centrifuging in hypertonic sea water are returned to normal sea water, they both take up water to about the same extent. The white half remains considerably smaller than when centrifuged in normal sea water.

6. The nucleus of the mature unfertilized egg increases perceptibly in hypotonic sea water and decreases in hypertonic sea water, to a greater percentage volume than the egg itself. It attains normal size on return to sea water.

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